Supplementary Materials

Methods

Antibodies and reagents

The following antibodies and chemicals were acquired: Anti-p16 (orb228122; Biorbyt, San Francisco, CA), anti-p90RSK (MAB 2056; R&D Systems, Minneapolis, MN), and anti-α-tubulin (T5168; Sigma-Aldrich, St. Louis, MO) were purchased. In addition, antibodies against TERF2IP (5433), anti-cleaved Caspase3 (9664), anti-FLAG (14793) were purchased from Cell Signaling Technology (Beverly, MA). Anti-intracellular adhesion molecule-1 (ICAM-1) (8439), anti-VE-cadherin (SC-48), and anti-VCAM-1 (H-276, SC-8304) were from Santa Cruz (Santa Cruz, CA) and anti-TRF2 (ab108997) were from Abcam (Cambridge, MA); anti-VCAM-1 (NBP1-4749) was from Novus Biologicals (Littleton, CO); anti-platelet EC adhesion molecule-1 (PECAM-1) (550274) was from BD Biosciences (Franklin Lakes, NJ). Protease inhibitor cocktail (p8340), PMSF (36978), and NEM (E3876) were from Sigma-Aldrich (St. Louis, MO).

Recombinant Human RSK1 protein (992-KS) was purchased from R&D Systems (Minneapolis, MN). The lipid profile kit (ab65390) was from Abcam (Cambridge, MA); the iQSYBR Green Supermix (1708882) and iScript cDNA synthesis kits (1708890) were from Bio Rad (Hercules, CA); the TL PNA kit/FITC flow cytometry kit (K5327) was from Agilent Technology (Santa Clara, CA); the FITC annexin V apoptosis detection kit was from BD Biosciences (556547; Franklin Lakes, NJ); and the 8-hydroxy-2'-deoxyguanosine chemiluminescence detection reagent kit (NEL105001EA) was from PerkinElmer (Waltham, MA). We also purchased an anti-phospho-histone H2A.X (Ser139) antibody, clone JBW301, FITC conjugate (γ-H2A.X, 16-202A), and ApopTag peroxidase *in situ* apoptosis detection kit (S7100) from Millipore, (Burlington, MA), FITC conjugate mouse CD31 from BioLegend (102405, San Diego, CA), TaqMan reverse transcription reagents (N808-0234; Applied Biosystems, Foster City, CA), , a dual luciferase assay kit (E1910; Promega Life Sciences, Fitchburg, WI), and Lipofectamine 2000 transfection reagent (11668027; ThermoFisher Scientific, Waltham, MA).

Reagents
The RSK specific inhibitor FMK-MEA has been described previously (1).
Short interfering RNA (siRNA-Smart pool) targeted human TERF2IP *(*L-021219-00-0010) was purchased from Thermo Scientific Dharmacon. TRF2 siRNA was purchased from Santa Cruz (sc-3805). Non-specific siRNA negative control was purchased from Invitrogen (#12935-112).

Generation of plasmids and adenoviruses

Plasmids containing rat WT p90RSK1 (WT-*p90rsk*) (Genebank NM031107) and dominant-negative (kinase dead) *p90rsk1* with K94A/K447A mutations (DN-*p90rsk1*) were generated as we previously described(2). pLPC-human TERF2IP full length **(**#12542**)** was from Addgene. pCMV-Flag-TERF2IP-WT was obtained by subcloning TERF2IP from pLPC-human TERF2IP full length into pCMV-Tag2B vector (Agilent Technologies, Santa Clara, CA, USA) at sites recognized by the restriction enzymes *Eco*RI and *Xho*I. pCMV-Flag-TERF2IP-S205A mutant was generated by site-directed mutated pCMV-Flag-MAGI1-WT using a QuikChange site-

directed mutagenesis kit (Agilent Technologies) according to the manufacturer's instructions. FLAG-tagged adenoviral vectors containing TERF2IP-WT and -S205A (Ad-Flag-TERF2IP-WT and -S205A) were generated by cloning each corresponding insert from pCMV-Flag-TERF2IP-WT and -S205A into the pENTR1A vector (Life Technologies) at sites recognized by the restriction enzymes *Kpn*I and *Not*I, and then a recombinase reaction was performed to get a pDEST-based vector following manufacture's instruction (#K4930-00, ViraPower Adenoviral Expression System, Promega). pACT-TERF2IP (133-191 aa), -(1-282 aa) and full length were obtained by subcloning TERF2IP from pENTR-Flag-TERF2IP-WT into pACT vector at sites recognized by the restriction enzymes *BamHI* and *Xba*I. All constructs were verified by DNA sequencing by using vector specific primers. Where indicated, adenovirus containing β-galactosidase (Ad-LacZ) or green fluorescent protein (Ad-GFP) were used as a control.

Generation of human anti-phospho-TERF2IP-S205.

An antibody against phosphorylated TERF2IP S205 was made by Pierce Biotechnology Inc.: A peptide corresponding to amino acids (DAPVSP (pS205)SQKLKR) of human TERF2IP sequence was synthesized. Rabbits were immunized with the synthesized peptide at 1:1 ratio of saline and adjuvant (maximum 1 mL was used). The initial inoculation was via subcutaneous routes, and following injections were spread out into a minimum of 4 different sites to avoid any lesion formation. After the primarily immunization, the rabbits were checked for any adverse reactions including lesion formation, loss of appetite, and non-responsiveness. Once the rabbits passed the initial evaluation, boosts were given at week 2, 4, 6, 12, 18, 21, and 28 after the initial inoculation. At the time of each boost, serum was collected and tested for the presence of specific antibodies by Elisa. At the end of immunization process, the rabbits were euthanized and the entire serum was collected, absorbed with the same peptide with non-phosphorylated S205, and affinity purified.

Disturbed flow

We performed disturbed flow experiments as we reported previously(3) In brief, we used cones with radial grooves that were 1-mm deep. We showed tracks of fluorescent beads suspended in culture media when grooved and nongrooved cones were rotated at the same speed. Although the non-grooved cone created straight unidirectional tracks indicating steady laminar flow, tracks made by the grooved cone were short and not oriented in the same direction, indicating non-laminar (turbulent) movement of the media in the dish. Furthermore, we confirmed the ECs cell shapes under grooved cone becomes cobble stone-like shapes, also supporting that this system can recapitulate the condition observed under disturbed flow in vivo.

Immunofluorescence staining

Immunofluorescence staining was performed on paraffin sections, as described previously(4). In brief, the tissue sections were de-paraffinized, and heat-induced epitope retrieval was performed by boiling them in heat-induced epitope retrieval buffer containing 10 mM sodium citrate and 0.05% Tween 20 (pH 6) for 20 min. The slides were first blocked by being incubated with 10% normal goat serum for 60 min at RT and then incubated with primary antibodies (rabbit anti-VCAM 1:200 dilution, rabbit anti-VE-cadherin 1:200 dilution, or rabbit anti-cleaved caspase-3

1:300 dilution or rabbit IgG) at 4°C overnight, followed by incubation with Alexa Fluor 546-conjugated goat anti-rabbit IgG (1:2000 dilution) for 60 min at RT. The expression levels of VCAM1 and cleaved caspase 3 were imaged on an Olympus FX1200 MPE confocal laser scanning microscope.

Quantification of immunofluorescent intensities

We quantified the immunofluorescent intensities of the images using ImageJ analysis. First, we selected the region of interest (we quantified the entire confocal image captured at 40X from at least 3 different fields/animal) using the selection tool located on the ImageJ selection toolbar. The l-flow and d-flow areas within the aorta were identified on the basis of published and generally accepted anatomical locations where such flow patterns are known to occur, as we and others reported previously(3, 5-7). A typical l-flow area is located in the greater curvature area(6), which is also known as a high wall shear stress area. A d-flow area is the lesser curvature area, which is also a low wall shear stress area, as described previously(3, 5-7). As we reported previously (8), we also used the EC shape, outlined by anti-VE-cadherin staining, to identify l-flow areas (elongated cell shape) and d-flow areas (irregular cell shape). We adjusted the color threshold of the region of interest (Image\Adjust\Color Threshold). From the Analyze menu, we selected "Set Measurements", and then selected the field, min and max gray values, integrated density, and mean gray value. Finally, we selected "Measure" and read the values (integrated density) from a popup box with a stack of values for those regions of interest. The same procedure was performed for regions next to regions of interest that had no fluorescence. This served as the background. The corrected total region fluorescence was calculated as integrated density - (mean fluorescence of selected fields - background readings).

En face staining of mouse aortas

We performed *en face* staining of mouse aortic arches at the greater curvature area, as described in our previous reports(2, 3, 8).

Serum lipid profile analysis

Mice that had fasted overnight were euthanized with CO_2. Whole blood was collected in a 1.5-mL tube, allowed to clot for 45 min at RT, and centrifuged at 1,500 xg for 30 min at 4ºC. The cholesterol levels (high- and low-density lipoprotein) were determined using cholesterol assay kits (cat #EHDL-100, Bioassay System, USA).

Isolation of mouse lung endothelial cells (MLECs)

MLECs were isolated as described previously(2). One day before cell isolation, anti-PECAM-1-conjugated Dynabeads were prepared by first conjugating the beads with sheep anti-rat IgG (cat. no. 11035; Invitrogen, Carlsbad, CA, USA) and then incubating them with rat anti-mouse PECAM-1 (purified rat anti-mouse CD31, #553370; BD Pharmingen, San Diego, CA, USA) in a cold room overnight following the manufacturer's instructions. Whole animal perfusion was performed using phosphate-buffered saline (PBS). Lungs were harvested, washed thoroughly in cold PBS, minced finely with scissors, and were then digested in collagenase (2 mg/mL, #4177;

Worthington Biochemical Corp., Lakewood, NJ, USA). The resulting lung tissue suspension was triturated 15-20 times using a 20-mL syringe attached to a cannula, avoiding frothing, and was filtered through a cell strainer with 70 μm pore size. The crude cell preparation was pelleted and resuspended in cold Dulbecco's PBS with Ca/Mg (#SH30264.01; HyClone Laboratories) and 0.1% bovine serum albumin (#A9576; Sigma-Aldrich, St. Louis, MO, USA). The cell suspension was then incubated with anti-PECAM-1-conjugated Dynabeads (35 μL beads/mL cell suspension) at room temperature for 30 min with end-over-end rotation. After incubation, cells bound to the beads were recovered using a magnetic separator stand (#Z5410; Promega, Madison, WI, USA), washed with DMEM containing 20% FBS, and then suspended in a complete culture medium (DMEM containing 20% FBS supplemented with 100 μg/mL porcine heparin, 100 μg/mL ECGS (#2759; Sigma-Aldrich), MEM nonessential amino acids [#25025Cl; Corning], 1 mM sodium pyruvate [#15323581; Corning], and 1% P/S and plated in 0.2% gelatin-coated Petri dishes. When the cells reached 70-80% confluence, they were detached with trypsin-ethylenediaminetetraacetic acid to generate a single-cell suspension, pelleted, resuspended in Dulbecco's PBS with Ca/Mg and 0.1% bovine serum albumin, and incubated with anti-PECAM-1–coated beads as described previously(9). The cells bound to beads were collected, washed, cultured in a complete culture medium, and used for experiments when they became confluent. Cells were used for experiments at passages 3.

Isolation of mouse aortic endothelial cells (MAoECs)

Mice, 8–12 weeks old, were anesthetized and euthanized by rapid cardiac excision. The whole aorta was carefully excised and placed in DMEM supplied with 20% FBS. The aortas were cleaned of excess fat and connecting tissues. The aortas were minced into 1 mm fragments, and dispersed in PBS with 100 mM $CaCl_2$ and $MgCl_2$ containing 5 mg/mL collagenase type 2 (Worthington Biochemical, Lakewood, NJ, cat. no LS004174), 2 mg/mL glucose, and 30 U/mL DNAse I (Worthington Biochemical, cat. number LS006331) under constant agitation 45 min (180 rpm at 37°C). Enzyme activity was stopped by the addition of a 10% solution of FBS in PBS. Any remaining clumps of cells were dispersed by forcing through a sterile 18 G needle 20 times. The resulting mixture was filtered through and filtered through a 40 μm cell strainer (BD Biosciences, San Jose, CA, USA, cat. no 352340) and the single-cell suspension was washed twice in DMEM with centrifugation at 400 × g for 6 minutes. The final cell pellet was re-suspended in 6 ml of DMEM. The cell suspension was then incubated with anti-PECAM-1-conjugated Dyna beads (35 μL beads/mL cell suspension) at room temperature for 30 min with end-over-end rotation. After incubation, cells bound to the beads were recovered using a magnetic separator stand (#Z5410; Promega, Madison, WI, USA), washed with DMEM containing 20% FBS, and then suspended in a complete culture medium (DMEM containing 20% FBS supplemented with 100 μg/mL porcine heparin, 100 μg/mL ECGS (#2759; Sigma-Aldrich), MEM nonessential amino acids [#25025Cl; Corning], 1 mM sodium pyruvate [#15323581; Corning], and 1% P/S and plated in 0.2% gelatin-coated Petri dishes and used for experiments when they became confluent. Cells were used for experiments at passages 2.

Human endothelial cells culture

Human umbilical vein ECs (HUVECs) were obtained from collagenase-digested umbilical cord veins(10) and collected in M200 medium supplemented with LSGS (Cascade Biologics, Inc.,

Portland, OR) and 2% FBS (Atlanta Biologicals, Inc., Lawrenceville, GA). HUVECs were cultured in 0.2% gelatin pre-coated dishes and used in experiments between 3 and 7 passages. Human aortic endothelial cells (HAECs) were isolated from aortic explants of heart transplant donors of anonymous origin through the UCLA transplant program as previously described (11). Our HAEC population was derived from aortic explants of anonymous heart transplant donors. Therefore, we have no individual information, including ethnicity, history, or disease status(12). HUVECs and HAECs were cultured in Petri dishes or flasks coated with 0.2% gelatin type A (#901771; MP Biomedicals, Santa Ana, CA, USA), in Endothelial Cell Medium (ECM, #1001, ScienCell, Carlsbard, CA. USA) containing 465 mL of basal medium, 25 mL of fetal bovine serum (FBS, #0025, ScienCell, Carlsbard, CA, USA), 5 mL of Endothelial Cell Growth Supplement (ECGS, #1052, ScienCell, Carlsbard, CA, USA) and 5 mL of penicillin/streptomycin solution (P/S, #0503, ScienCell, Carlsbard, CA, USA). HAECs with less than 15 passages were used in this study.

Characterization and selections of ECs

Cells were grown on cover slips in a 6 well plate. Cells were washed two times with PBS and then fixed with cold methanol for 10 min at -20°C. After that cells were incubated with blocking buffer (1% FBS in PBS) for 30 min at RT. Cells were incubated with FITC-anti-CD31 (2.5 mg/mL) were incubated with the cells overnight at 4°C. After incubation cells were washed with three times with wash buffer (0.1% BSA in PBS), mounted with gold antifade, photographed using a microscope. Primary ECs with passage 4 can response to flow. Since the recourses of HAECs are limited, we used HAECs for the experiments to perform microarray analysis (Supplementary Fig.1) and detect the role of p90RSK and TERF2IP in EC senescence (Fig.3A-C). Since we found the similar tendency of TERF2IP-mediated EC activation and senescence between HAECs and HUVECs (Supplemental Fig.1D and E, Supplemental Fig.2A, Fig.1J, and Fig. 4C), we performed detailed mechanistic analysis to investigate the role of TERF2IP in EC biology, we used HUVECs. We also compared the d-flow-induced 8-oxo-dG induction in bovine aortic endothelial cells and HUVECs in Fig.1A and B, and also found the similar tendency of KD-p90RSK-mediated inhibition on d-flow-induced 8-oxo-dG, also suggesting that HUVECs can recapitulate the d-flow-mediated biology in ECs isolated from aorta.

Transfection and transduction

ECs were transfected with siRNA molecules at a final concentration of 100 nM or appropriate reporter plasmids or plasmid DNAs using Lipofectamine 2000 transfection reagent, according to the manufacturer's instructions. After transfection, cells were allowed to recover in the complete medium for 24-48 h. For adenovirus transduction, we used 20 multiplicities of infection for each adenovirus. Prior to use, cells were cultured overnight in a low-serum (1% FBS) medium.

Western blotting analysis

ECs were washed twice with cold PBS, and whole cell lysates were prepared in RIPA buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1% Nonidet P40, 0.1% SDS, 1 mM dithiothreitol, 1:200-diluted protease inhibitor cocktail [P8340, Sigma-Aldrich], and 1 mM PMSF). Total lysates were resolved by SDS-PAGE and electrotransferred onto a Hybond

enhanced chemiluminescence nitrocellulose membrane, which was then incubated with antibodies against each of the proteins to be detected in the lysate. Bound antibodies were visualized using enhanced chemiluminescence detection reagents (Amersham Pharmacia Biotech) according to the manufacturer's instructions and quantified by densitometry using ImageJ software. Tubulin was used as the loading control and was always probed together with the specific protein of interest. The immunoblotted band intensities of the proteins of interest were standardized against the intensity of the anti-tubulin band. In most cases, anti-tubulin immunoblots were not shown in each figure to save space.

CheckMate Mammalian Two-Hybrid assay

A dual-luciferase reporter assay system (#E1960; Promega)(2, 13) was used to study p90RSK-TEFR2IP interaction, in addition to a co-IP approach. ECs were co-transfected with a full-length human TERF2IP fused to the activation domain VP16 (pACT-TERF2IP), a full-length p90RSK fused to the Gal4-binding domain (pBIND-p90RSK), and a Gal4-responsive luciferase reporter (pG5-Luc). In some cases, the transfection mixture also contains pACT-TERF2IP-Myb or control vector. After 24 h of transfection, cells were harvested in 1X passive lysis buffer (cat. no. E194A, Promega), and luciferase activity in resulting cell lysates was measured using a GloMax 20/20 Luminometer (Promega). The increase in luciferase activity, which was calculated by normalizing firefly luciferase activity according to Renilla luciferase activity (ratio of firefly luciferase activity to Renilla luciferase activity), indicates the binding of p90RSK with TERF2IP.

Supplemental Figures

Supplemental Figure 1. Depletion of TERF2IP inhibits the inflammation-related signaling and expression of genes and adhesion molecules in ECs exposed to d-flow

(**A**) After 48 h of a control siRNA or TERF2IP siRNA transfection, HAECs were subjected to d-flow for 16 h, and expression levels of TERF2IP-mRNA were analyzed by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 8-9. ** p <0.01, *p <0.05. (**B**) Differential regulation of canonical pathways in human aortic endothelial cells (HAECs) treated with control or TERF2IP siRNA was analyzed using Ingenuity Pathway Analysis (-log[p]>4). The bar chart indicates the -log(p) value of the significance of enrichment. The line represents the ratio of differentially expressed genes from the microarray data set to the genes present in each canonical pathway. Positive z-score predicts increased pathway activity; negative z-score predicts decreased pathway activity. (**C**) The regulator effects algorithm (consistency score, 14.311) was determined by gene enrichment analyses (right-tailed Fisher exact test p-value <0.001, absolute value of z-score >2.0) according to the Ingenuity Pathway Analysis upstream regulator and downstream effects analysis (we only included categories of cardiovascular disease and cardiovascular system development and function) in our microarray data set. Light blue symbols denote genes with lower expression, and dark blue symbols denote the predicted inhibition of upstream regulator networks and adhesion of ECs as downstream functions or disease categories affected by the expression change in our data set (shown in the middle tier) in TERF2IP siRNA-treated HAECs compared with in control siRNA-treated cells. The arrows with blue solid lines indicate direct (usually physical) inhibitory interactions between 2 molecules in the direction of the arrow, whereas arrows with blue dashed lines denote indirect inhibitory interactions (e.g., molecule/gene A affects molecule/gene B). The yellow line shows that the findings are inconsistent with the state of the downstream molecule. The abbreviations are defined in Supplementary Table 1. (**D**) HUVECs (left) and HAECs (middle and right) were transfected by control siRNA and TERF2IP siRNA for 24 h (together with NF-κB reporter gene). After 24 h of NF-κB reporter gene transfection, cells were subjected to d-flow (left) or TNF-α (TNF, 10 μg/mL, right) for 16 h, and NF-κB activity was measured by a luciferase assay. Data represent mean ± S.D., n = 5. **p <0.01, *p <0.05. (**E**) After 48 h of control siRNA or TERF2IP siRNA transfection, HAECs were subjected to d-flow for 16 h, and expression levels of VCAM1, E-selectin, ICAM1 and TNFα mRNA were analyzed by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 9. **p <0.01, *p <0.05. All statistical analyses in this figure were done by 1-way ANOVA followed by Bonferroni post hoc test.

Supplemental Figure 2. TERF2IP S205 phosphorylation plays a crucial role in EC activation.

(**A**) After 48 h of control siRNA or TERF2IP siRNA transfection, HUVECs were subjected to d-flow for 16 h, and expression levels of VCAM1, E-selectin, ICAM1 and TNFα mRNA were analyzed by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 3. **p <0.01, *p <0.05. **(B**) HUVECs were transfected with NF-κB reporter gene and also with either TERF2IP WT or the S205A mutant for 24 h and incubated with TNF-α (10 μg/mL) for 24 h. NF-κB activity was detected by luciferase assay. Data represent mean ± S.D., n = 5. (**C**)

HUVECs were transduced by Ad-TERF2IP S205A or Ad-TERF2IP WT for 24 h and exposed to d-flow for 16 h. Expression of VCAM1, ICAM1, and E-selectin mRNAs was determined by quantitative real-time polymerase chain reaction. Data represent mean ± S.D., n = 3-5, **p <0.01, *p <0.05. All statistical analyses in this figure were done by 1-way ANOVA followed by Bonferroni post hoc test.

Supplemental Figure 3. Both senescence and expression of inflammation-related molecules induced by overexpression of wild-type p90RSK are inhibited by depletion of TERF2IP in mouse aortic endothelial cells (MAoECs).

(**A, B**) MAoECs were isolated from WT or *Terf2ip*^{homo}-endothelial cell-specific knockout mice. After transduction with Ad -p90RSK-WT or Ad-GFP as a control for 12 h, expression of certain proteins was analyzed by Western blotting using specific antibodies as indicated (**A**). Graphs represent densitometry data of immunoblots for selected proteins. Data represent mean ± S.D., n = 3. **p <0.01, *p <0.05 by 1-way ANOVA followed by Bonferroni post hoc test. (**C**) Purity of cultured mouse aortic endothelial cells (MAoECs) is demonstrated by immunofluorescence staining of CD31, and we observed that most of the cells were CD31 positive. To quantify the purity of the MAoECs, we also employed the cells to flow cytometric analysis by CD31 staining. We isolated ECs from 6 mice per group, and the purity of CD31 positive cells (%) were 76 ± 2.8 (isolated from WT) and 73 ± 1.4 (isolated from Terf2ip^{homo}-EKO mice), mean ± S.D., n = 2 per group.

Supplemental Figure 4. Cholesterol levels and body weight in NLC/*Ldlr* $^{-/-}$and *Terf2ip*^{het}-endothelial cell-specific knockout mice /*Ldlr*$^{-/-}$

(**A**) High- and low-density lipoprotein (HDL and LDL) levels after 8 weeks of a high-fat diet in wild type (NLC/*Ldlr*$^{-/-}$) or *Terf2ip*^{het}-endothelial cell-specific knockout (HET)/*Ldlr*$^{-/-}$ mice are shown. (**B**) Body weight (BW) of NLC/*Ldlr* $^{-/-}$or *Terf2ip*^{het}-endothelial cell-specific knockout (HET)/*Ldlr*$^{-/-}$ mice by age are shown.

Supplemental Table 1. Abbreviations used in Supplemental Fig. 1C.
The activation of granulocytes as the highest scoring regulator effect network on the basis of the results of a right-tailed Fisher exact test (consistency core; 598.131, Supplemental Fig.1C). Expr Log Ratio: Log2 ratio differential expression.

Supplemental Table 2. List of qRT-PCRT primers.

Primers	Sequences
h-GAPDH-F	5'-GGT GGT CTC CTC TGA CTT CAA CA-3'
h-GAPDH-R	5'-GTT GCT GTA GCC AAA TTC GTT GT-3'
h-VCAM1-F	5'-CCG GAT TGC TGC TCA GAT TGG A-3'
h-VCAM1-R	5'-AGC GTG GAA TTG GTC CCC TCA-3'
h-ICAM1-F	5'-GTC CCC TCA AAA GTC ATC C-3'
h-ICAM1-R	5'-AAC CCC ATT CAG CGT CAC C-3'
h-ESELECTIN-F	5'-GCT CTG CAG CTC GGA CAT-3'
h-ESELECTIN-R	5'-GAA AGT CCA GCT ACC AAG GGA AT-3'

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